A retrospective study of chromosomal microarray analysis in the diagnosis of patients with dysmorphic features, intellectual disability, developmental delay, congenital abnormalities

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ABSTRACT

Copy number variations (CNVs) are defined as genomic imbalances with loss or gains in chromosomal material. Chromosomal microarray analysis (CMA) is an effective method for detecting CNV variants. Here, we report our laboratory’s array data on patients with dysmorphic features, intellectual disability, developmental delay, and congenital abnormalities. Microarray analysis of 1070 patients was prepared using Agilent ISCA v2 Human Genome 8x60k oligonucleotide array and Illumina Infinium HumanCytoSNP-12 v2.1 iScan SNP array. CNV classification was made according to variant size and type, gene content, and effect on phenotype. CNVs were detected in 295 of 1070 patients . %74 of patients had normal results, %17 of patients had pathogenic and % 9 of patients had VUS (variant of unknown significance) variants. A total of 332 CNVs were found in 295 patients. %57.2 (180/332) of CNVs were classified as pathogenic, %4.2 (14/332) of CNVs as likely pathogenic, and %41.5 (138/332) of CNVs as VUS. The average size of CNVs was 6.4 Mb, with a range of 52 kb to 73.2 Mb. Most of the pathogenic variants were 1-5 Mb. 70% of pathogenic variants were losses. Most of the VUS variants were gains (53.6%). 50.2% of pathogenic variants were well-defined CNV syndromes. The smallest pathogenic variant was 52 kb. Overall, the most common CNV syndrome was 22q11 deletion syndrome. Microarray analysis is currently used as an effective diagnostic tool in the diagnosis of patients with congenital and developmental abnormalities, and intellectual disability especially those with unexplained phenotypes. The presence of these clinical features will be an important criterion in the selection of suitable patients for microarray analysis.

Keywords: microarray, CNV syndrome, copy number variants, microdeletion, microduplication

Introduction

Intellectual disability, developmental delay, and congenital abnormalities especially unexplained phenotypes have been screened for the presence of copy number variants [1, 2]. Cytogenetic studies have an important role in the diagnosis of several phenotypes, such as congenital anomaly, developmental retardation, intellectual disability, and autism [1, 2, 3]. Therefore, genetic evaluation should definitely be included in the diagnosis of patients with these features especially when underlying causes are unclear. Conventional chromosomal analyses (G-banded karyotype) are unable to detect submicroscopic aberrations below 5-10 Mb. Submicroscopic chromosomal abnormalities can be detected using genomic microarrays which are used to detect copy number variants (CNV). A chromosomal region of at least 1 kb in size that differs compared to the reference human genome is defined as a copy number variant [4]. Microarray-based comparative genomic hybridization and single-nucleotide polymorphism-based microarray platforms are commonly used in clinical practice and provide genome-wide analysis of genomic imbalances with a higher resolution compared to conventional cytogenetic analysis. Due to its higher diagnostic capacity, microarray analysis is highly preferred in the diagnosis of patients with intellectual disability, neurodevelopmental disorders, and/or congenital anomalies and is recommended as the first-tier diagnostic test for these patients5. The detection rate of microarray analysis is reported between 12 and 20%, depending on the patient selection and array platforms used [5, 6].

In this study, we reviewed the CMA results of 1070 patients with unexplained phenotypes like dysmorphic features, intellectual disability, developmental delay, and congenital abnormalities. We aimed to find the distribution of these particular phenotypes in patients with genetic abnormalities, which are detected by CMA analysis and to analyze the clinical array data of patients, in order to identify whether some clinical features are more linked to pathogenic variants or not.

Material & Methods:

Patients

This study involved 1070 patients (608 males and 462 females, age range from 1 month to 19 years old, average of 5 years old) with dysmorphic features, intellectual disability, developmental delay, and congenital abnormalities (referred to our laboratory from January 2017 to January 2019). Conventional G-banded karyotype analyses were performed for all patients to detect numerical and structural chromosomal abnormalities. Patients with aneuploidy were not included in this study. The approval of the Ethics Committee (Ethical Committee of Yuksek Ihtisas University, Faculty of Medicine /IRB reference number 2020/02/03) was obtained.

CMA Analysis

Genomic DNA was extracted from patients using a DNA isolation kit (QIAamp DNA Blood Mini Kits, Qiagen, Hilden, Germany). The quality and quantity of DNA samples were evaluated using the NanoDrop™ 2000(Thermo Fisher, USA).

The isolated DNA samples were studied on two different array platforms; detection of genomic CNVs was performed with Agilent Sure Print G3 Human CGH Microarray, 8x60K (Agilent, USA) and Illumina Infinium HumanCytoSNP-12 v2.1 iScan SNP array, according to the manufacturer’s instructions. 47.8% (512/1070) of patients were analyzed by Agilent Sure Print G3 Human CGH Microarray, 8x60K (Agilent, USA). Sure Print G3 Human CGH Microarray 8x60K slide with 62.976 probes and an average of 41 kb probe interval were used on this platform. Array data were visualized and analyzed by Agilent Cytogenomics (version4.0.3.12) Software (Agilent, USA).

52.2 % (558/1070) of patients were analyzed by Illumina iScan SNP array. A microarray study was done on Illumina iScan platform with Illumina Infinium HumanCytoSNP-12 v2.1 chip. Genotyping of approximately 299,140 single nucleotide polymorphisms (SNPs) was performed throughout the entire genome. The median range of Illumina microarray chip probes is 6.2 kb. Copy number variations were detected and visualized through GenomeStudio (v.2.0.4) and CNV Partition (v.3.2.0) analysis programs (Illumina). The data were analyzed with BlueFuse Multi Software. The size threshold for CNV analysis was set at > 200 kb for gains and > 100 kb for losses.

All copy number variants (CNV) were classified according to the criteria defined in American College of Medical Genetics (ACMG) guidelines7. ACMG recommends five categories; pathogenic, likely pathogenic, variant of uncertain significance, likely benign, and benign for variant classification [7]. CNVs are classified according to the following criteria; pathogenic CNVs; variants containing disease-causing (morbid) OMIM genes, large deletions or duplications (usually>3 Mb), variants containing CNV syndrome defined in that region, variants reported in the literature and databases as pathogenic or likely pathogenic. VUS CNVs; are variants without sufficient evidence of whether they are pathogenic or benign, variants reported in the literature and databases as Class 3 (VUS). The likely benign and benign CNVs were not reported and the patients with benign variants were evaluated as normal. Furthermore only possible clinically relevant VUS variants were mentioned in the study. The classification of variants was done using the following databases; Database of Genomic Variants (DGV - http://projects.tcag.ca/variation/), Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER - http: // decipher.sanger.ac.uk/) and UCSC Genome Bioinformatics database (http://genome.ucsc.edu), Online Mendelian Inheritance in Man (OMIM- https://www.omim.org/). Genomic positions were reported with reference to GRCh37 / hg19 Human Genome Build 37.

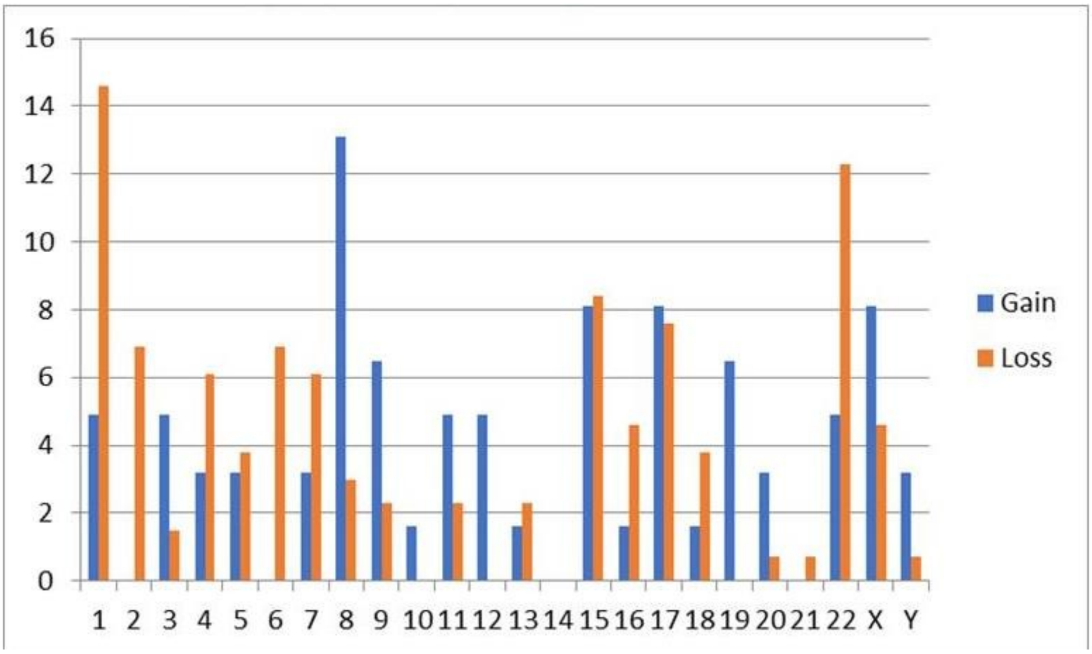
Results:

Of the 1070 patients, (56.9%) 609 males and (43.1%) 461 females were analyzed. The Illumina SNP array was used in 558 patients, and the Agilent Array was used in 512 patients.

In 295 of 1070 patients (27.5%), CNVs were detected. 74% of patients have normal results, 17% of patients have pathogenic and 9% of patients have VUS variants. A total of 332 CNVs were found in 295 patients. The average size of CNVs was 6.4 Mb, with a range of 52kb to 73.2 Mb. These CNVs were classified according to ACMG/ClinGen recommendations [7]. 57.2% (180/332) CNVs were classified as pathogenic, 4.2% (14/332) as likely pathogenic and 41.5% (138/332) as VUS. The percentage of pathogenic CNVs in loss (70%,127/180) was more than that in gain (30%, 53/180). Pathogenic CNVs in different sizes was classified as follows: 5.5% (10/180) loss and 0.5% (1/180) gain CNVs were between 300 kb and 500 kb; 5.5% (10/180) loss CNVs were between 500 kb and 1MB, 31% (56/180) loss and 5% (9/180) gain CNVs were between 1 Mb and 5 Mb; 15% (27/180) and 4.5% (8/180) gain CNVs were between 5 Mb and 10 Mb; 13% (24/180) loss and 20% (36/180) gain CNVs were >10 Mb(Table 1). Most of the pathogenic variants were between 1 Mb and 5 Mb. Figure 1 represents the frequency and number of pathogenic CNVs found per chromosome. The smallest pathogenic variant was 52 kb and caused Rubinstein Taybi Syndrome.

Figure 1. Chromosomal distribution of pathogenic/likely pathogenic CNVs identified in the present study.

Abbreviation: CNV, copy number variation



The percentage of VUS CNVs in gain (53.6%, 74/138) was more than that in loss (46.4%, 64/138). VUS CNVs in different sizes were classified as follows: 12.1% (17/140) loss and 7.8% (11/140) gain CNVs were >300kb. 6.4% (9/140) loss and 14.2% (20/140) gain CNVs were between 300 kb and 500 kb; 7.8% (11/140) loss and 12.1% (17/140/) gain CNVs were between 500 kb and 1MB, 10(14/140) loss and 16.4% (23/140) gain CNVs were between 1 Mb and 5 Mb; 1.4% (2/140) and 2.8% (4/140) gain CNVs were between 5 Mb and 10 Mb; 0.7%(1/140) gain CNVs were >10 Mb (Table 1).

Table 1. Distribution of pathogenic/likely pathogenic and VUS CNVs according to their size.

Abbreviations: VUS; variant of unknown significance,≠;number

Size of	≠ of	≠ of	≠ of likely	≠ of likely	≠ of	≠ of

imbalance	pathogenic gains	pathogenic losses	pathogenic gains	pathogenic losses	VUS gains	VUS losses
>10 Mb	36	24	2	1	1	
5-10 Mb	7	27			4	2
1-5 Mb	9	56	4	2	23	14
1 Mb-500 kb		10	1	4	17	11
500-300 kb	1	10			20	9
<300 kb					11	17
Total	53	127	7	7	74	64

Table 2. Distribution of patients and their pathogenic/likely pathogenic and VUS CNVs according to clinical features.

Abbreviations: P; pathogenic, LP;likely pathogenic, VUS; variant of unknown significance,≠;number, chromosome [number of CNV]

Clinical features	**≠ of patients with P/LP CNVs**	**Chromosomal distribution of P/LP losses**	**Chromosomal distribution of P/LP gains**	**≠ of patients with VUS CNVs**	**Chromosomal distribution of VUS losses**	**Chromosomal distribution of VUS gains**
Dysmorphic features	68/182	1[13], 7[8],15[7],22[7], 4[3], 5[3], 17[3], 2[2], 8[2], 13[2], 6, 9, 16, 18, Y	1[3], 4[2],8[2],3, 11,12,21,22, Y	30/96	17[3], 15[2],2,7,13, 21,22	15[3],1[2],17[2], X[2],10[2], 8[2],2,5,6,7, 9, 12
Intellectual disability	48/182	1[6], 15[5], 4[3],7[3], 17[3],2[2], 22[2], 5,6, 9,10,12,13, X	15[3],22[2], 3,7,9,10, 11,13,16,17, 18,20,21,X	20/96	12[3],2[2], 15,16,17,20, 22	X,2,5,7,10,12,14,15,16,17
Developmental delay	37/182	4[4], 15[4], 16[3], 6[2],7[2],8[2], 17[2], 1,2,9,12,13, ,19,18,22	20[2],3,7,14,15,17,19, 22,X	12/96	2,13,13	X[2], 17[2], 2,5,7,8,15,
Congenital heart anomalies	36/182	22[13],1[3],2[2],7[2],6,8, 13	1[3], 4[2],8[2],3, 11,12,21,22, Y	4/96	2,7,17,22	
Seizures/ epilepsy	17/182	1[3], 7[3], 16[2],2,4,5, 8,15,	X,7,15,17	16/96	2[2], 12[2],6, 13,14,15,22,X	17[2],1,2,10,X,
OTHER	16/182	1[4],6,11,12,17,22,X	7, 8,9,17,X	12/96	2[2],15,16,17	5[2],7,19,21, 22,X
Skeletal malformation	10/182	1[4],17,20	3,17,20	10/96	2[2], 1,9,14, 16	11,X,5,19
Neuromotor delay	9/182	7, 17	8[2],17[2],4, 9, X	2/96	15	7
Microcephaly	9/182	7[2],1,13,17	17[3],19			
Hypotonia/ muscle weakness	8/182	2,5[2],9,16,X		4/96	2,15,20	17
Multiple Congenital anomalies	6/182	1,8,9	4,22,X	2/96		12,X

Short stature	5/182	Y,1[2]	Y,6	2/96	1,15	
Learning disability	4/182	Y	17,X,Y	1/96	11,12,14	15
Speech delay	3/182	1,17	8	1/96		8
Autism	2/182		16,X	8/96	9,19	X,1,7[2],17[2]

Table 3.The summary of microdeletion/microduplicstion syndromes.

CNV SYNDROMES	NUMBER OF PATIENTS
Velocardiofacal/DiGeorge Syndrome	16
1q21.1 microdeletion syndrome	8
Angelman / Prader Willi Syndrome	7
Williams-Beuren Syndrome	6
3q29 microdupliction syndrome	4
22q11 duplication syndrome	4
Wolf-Hirschhorn syndrome	4
1q21.1 TAR (Thrombocytopenia-Absent Radius) Syndrome	4
16p11.2 Microdeletion syndrome	3
Smith-Magenis syndrome	3
Cri du Chat syndrome	3
Potocki-Lupski syndrome (17p11.2 duplication syndrome	2
15q11.2 BP1–BP2 microdeletion syndrome	2
1p36 microdeletion syndrome	2
Koolen-De Vries syndrome	2
2p15-16.1 microdeletion syndrome	1
STS deficiency/ichtiosis	1
Rubistein Taybi syndrome	1
2q37 microdeletion syndrome	1
Xq28(MECP2) duplication syndrome	1
WAGR 11p13 microdeletion syndrome	1
Y microdeletion	1
17q12 duplication syndrome	1
8p23.1 duplication syndrome	1
Xp11.22-p11.23 microduplication syndrome	1
ATR-16 syndrome (Alfa thalassemia/mental retardation syndrome type 1)	1
NF1- microdeletion syndrome	1
15q24 microdeletion syndrome	1
8p23.1 microdeletion syndrome	1
Distal 22q11.2 microdeletion syndrome	1
Mİller-Dieker Syndrome	1
15q26 over growth syndrome	1
1q21.1 recurrent microduplication	1
SHOX deletion	1

In our study, we found that chromosome 1 is more prone to genomic rearrangements than other chromosomes. Chromosomes 8, 22, 15, 17, and X had the highest frequency of carrying pathogenic CNVs after chromosome 1. Despite having a large percentage of CNVs and also increased genomic size, chromosome 2 CNVs were largely classified as VUS. Most of the VUS variants were found to be located on Chromosome 2. The percentage of referral reasons for the patients with pathogenic and VUS variants were described in Table 2. The most common referral reason in the patients with pathogenic and VUS variants was dysmorphic features (Table 2). In addition to dysmorphic features, intellectual disability and developmental delay were the most common clinical findings in patients with pathogenic variants.

Discussion:

Microarray analysis is commonly used as a first-tier test in the diagnosis of patients with intellectual disabilities, developmental delay, and multiple congenital anomalies. The detection rate of microarray analysis was reported between 12 and 20% in previous studies. Our results indicate that the detection rate of pathogenic variants is 17%. Mc Cormack et al reported the frequency of pathogenic variants as 8.6 % for postnatal cases [8]. The authors compared their results with the previous studies; the frequency of 8.6% compared to 6.9 %, which was reported by Shaffer et al., and 8.3 and 9.8%, which were reported by Park et al. and Ahn et al., respectively [9, 10, 11]. The differences between the detection rates of previous studies are depending on the population, methods, and variant classification guidelines used [6]. The International Standard Cytogenomic

Sequence (ISCA) Consortium has compiled the results of 33 microarray analysis studies involving a total of 21,698 patients, and according to the results of the review, the microarray analysis has a high sensitivity in the diagnosis of patients with unexplained developmental delay / mental retardation, autism spectrum disorder (ASD) or congenital anomalies, especially in the diagnosis of submicroscopic deletions and duplications [5]. It was observed that microarray analysis gave a higher chance of diagnosis compared to conventional cytogenetic (G-banding) analysis (microarray; 15% –20%, G-banding; ~ 3%) [3].

Most of the pathogenic variants (70%) were losses and this is consistent with previous studies that losses have higher pathogenicity than gains [12]. Exome Aggregation Consortium's data revealed that many gains are benign when compared to losses and most of the pathogenic variants were larger than VUS variants [13]. As we know from previous studies that when the size of the variant is larger, it contains more genes and the likelihood of being pathogenic becomes higher [5, 14]. Cooper et al stated that as CNV size increases, the risk of disease increases [15]. Cooper's study revealed that the carriers of CNVs larger than 3 MB had a disease risk with an odds ratio of nearly 50 and the carriers of CNVs larger than 1.5 MB had a disease risk with an odds ratio of>20. They found that patients with craniofacial abnormalities and congenital heart defects had larger CNVs than those with epilepsy and autism [15]. In the present study, most of the pathogenic copy number variants are larger than 1 Mb and the smallest size of pathogenic variants was 52 kb (deletion). Although this variant was below the detection limit of the platform we analyzed, the deletion was found to be significant because the deletion was compatible with the patient's phenotype and the number of probes covered by that area was sufficient.

We estimate that 85/334 of our variants (25.7%) were consistent with a known CNV syndrome (Table 3). 82/1070 (7.6%) patients have CNV syndrome and two different CNV syndrome were diagnosed in 2 patients (2/82) (patient 1; 3q29 microduplication syndrome, 22q11 duplication syndrome, patient 2; 22q11 duplication syndrome/16p11.2 microduplication syndrome). The most common CNV syndromes in our study were Velocardiofacal / DiGeorge Syndrome, 1q21.1 microdeletion syndrome, Angelman / Prader Willi Syndrome, Williams-Beuren Syndrome (Table 3). Wang et al also reported that Angelman / Prader Willi syndrome, Williams-Beuren syndrome, 22q11.2 microdeletion/microduplication syndrome are the most common CNV syndromes16. In our study, the most common detected syndrome was Velocardiofacial/DiGeorge Syndrome. That was consistent with the results of other aCGH studies [1, 17, 18].

Based on the medical records, the most frequent referral reasons were developmental delay, intellectual disability, and dysmorphic features. The same clinical features are predominant in other microarray studies [19, 20, 21, 22]. Chaves reported that congenital anomalies with facial dysmorphic features were more than 58% of their cohort and this frequency was similar to previous studies [20, 21]. Dysmorphic features, especially those associated with neurodevelopmental delay, play an important predictive role for pathogenic CNVs [23]. Dysmorphic features, developmental delay, intellectual disability, and congenital heart defects were the most common clinical findings in the patients with pathogenic variants, respectively (Table 3). Several studies, with different selection criteria, were described specific phenotypes related to pathogenic variants [3, 6, 19, 24]. In some of these studies, dysmorphic facial features are one of the most frequently associated clinical findings with pathogenic CNVs23. Manini et al reported that 55.6% of patients with neurodevelopmental delay are associated with multiple congenital anomalies and/or dysmorphic features and/or epilepsy. Fan et al found that facial dysmorphism and congenital heart defects were more associated with pathogenic CNV findings than epilepsy and autism6. In this study, the detection of CNV is high in patients with congenital heart defects, microcephaly, hypotonia, facial dysmorphism, and developmental retardation / mental retardation [6]. Miller et al summarized the data of clinical CMA studies (21,698 Subjects) and according to this summary, developmental delay and intellectual disability were the most common phenotypes in patients. Miller et al suggested that the microarray should be a first-tier test for the diagnosis of patients with developmental delay/intellectual disability with or without additional major congenital anomalies or dysmorphic features [5]. Lee et al reported a cohort of 649 patients with developmental delay or intellectual disability and the diagnostic yield was 16.9%. They found that 110 patients had pathogenic CNVs and most of them were deletions (76.3%) [25]. Previous studies postulated that as the severity of ID increased, the diagnostic yield of CMA increased [6, 15, 26]. Several studies report a 20%–25% detection rate of pathogenic CNVs in children with moderate or severe intellectual disability [27].

Congenital heart defects were one of the most common clinical features in patients with pathogenic variants in our study. Congenital heart defects have already been reported as linked to pathogenic CNVs [23]. According to Maini's study, VSD (ventricular septal defect) was described as an independent predictive value for pathogenic CNVs. They reported that 16.9% of patients with VSD have pathogenic CNVs [23]. Previous studies showed that the comorbidity of congenital heart defects (CHD) in unexplained developmental delay/intellectual disability is the strongest phenotype associated with pathogenic CNV finding [6, 28]. Geng et al. also reported that the detection rate of pathogenic CNVs in congenital heart disease patients with co-occurring developmental delay / intellectual disability or autism was found 22.7%[29]. Fan et al. reported a retrospective study that included genotype-phenotype correlation with the array data of 710 patients with a history of developmental retardation/mental retardation [6]. The percentage of pathogenic CNVs was found to be significantly higher in the subgroups with congenital heart defects accompanying developmental retardation/mental retardation.

In the present study, the detection of pathogenic and VUS CNVs was high in patients with dysmorphic features, intellectual disability, and developmental delay. While the most common clinical findings in patients with VUS variants are developmental delay and intellectual disability, the least rated finding is microcephaly.

Conclusion:

The pathogenic CNV detection rate for CMA in this study was 17%. Our study emphasizes the importance of detecting copy number variants in the genetic diagnosis of patients with referring indications, such as congenital anomalies, dysmorphic features, developmental delay, and intellectual disability. Our results suggest that the presence of dysmorphic features, developmental delay, and intellectual disability could lead to deciding to perform CMA as the first-tier test. The accumulation of results obtained from studies similar to our research will be helpful in revealing the underlying pathogenic cause and establishing the genotype-phenotype correlation in patients with similar clinical findings. Several retrospective studies have been reported to detect clinical features as the predictive factors of pathogenic CNVs in patients with unexplained intellectual disability, developmental delay, and dysmorphic features. A better understanding of predictive clinical features of pathogenic variants will help us to improve the diagnostic yield of CMA.

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Disclosure Statement:

The authors have no conflicts of interest to declare.

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